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13. ABSTRACT (Maximum 200 Words)

Neurofibromatosis type 2 (NF2) is associated with a homozygous inactivation of the neurobibromatosis 2 gene (NF2). Despite intense study of the NF2 gene, the mechanism by which the NF2 tumor suppressor acts to prevent tumor formation is not well understood. The NF2 transcript undergo alternative splicing, generating a series of mRNA isoforms lacking one or more exons. Presently, the role of alternative splicing of NF2 mRNA is no understood. The NF2 transcripts are also terminated at different polyadenylation sites. The role of this differential polyadenylation is not known. The goal of this research is to examine the role of posttranscriptional regulation (alternative splicing and differentiation polyadenylation) of the NF2 gene. During this reporting period, we have found that the pattern and relative frequency of alternatively spliced NF2 isoforms expressed in vestibular schwannomas appear to be different from those detected in other human cell types. *In vitro* analysis showed that the two schwannoma-expressed NF2 cDNA isoforms lacking exons 15 and 16, or exons 8 and 16 did not possess growth inhibitory activity in human 293 cells. Experiments are in progress to address whether these NF2 isoforms preferentially expressed in schwannomas possess any properties conducive to tumor formation *in vivo*.

14. SUBJECT TERMS
Neurofibromatosis 2 (NF2), Vestibular Schwannomas, Schwann Cells, NF2 Gene, Transcript, RNA, Posttranscriptional Regulation, Alternative Splicing, cDNA Isoform, Differential Polyadenylation

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Table of Contents

INTRODUCTION:

Neurofibromatosis type 2 (NF2) is associated with a homozygous inactivation of the neurofibromatosis 2 gene (*NF2*), which encodes a protein named 'merlin' for moesin-ezrin-radixin like protein (Trofatter et al., 1993). Despite intense study of the *NF2* tumor-suppressor, the mechanism by which merlin acts to prevent tumor formation is not well understood (reviewed in Gusella et al., 1999; Gutmann, 2001). The *NF2* transcripts undergo alternative splicing, generating a series of mRNA isoforms lacking one or more exons. Presently, the role of alternative splicing of *NF2* mRNAs is not understood. *NF2* isoform 1 (without exon 16) but not isoform 2 (containing all 17 exons) possess growth inhibitory properties (Gutmann et al., 1999). Also, transgenic mice over-expressing the *NF2* isoform with a deletion of exons 2 and 3 in Schwann cell lineage showed a high prevalence of Schwann cell hyperplasia and tumors (Giovannini et al., 1999). These results raise the possibility that functional contribution of the *Nf2* tumor suppressor may require a balanced expression of various isoform proteins in Schwann cells and/or other cell types. In addition, we found that differential usage of multiple polyadenylation sites also contributes to the complexity of human *NF2* transcripts (Chang et al., 2002). Presently, the role of differential polyadenylation of *NF2* transcripts is not known. The goal of the proposed research is to examine the role of posttranscriptional regulation (alternative splicing and differentiation polyadenylation) of the *NF2* gene. Ultimately we hope to provide a better understanding of the mechanisms of NF2 tumorigenesis.

BODY:

Aim 1: Analysis of the Expression Pattern of Alternatively Sliced *Nf2* Transcripts in Schwann Cells and Vestibular Schwannomas.

Task 1: We have closely worked with Dr. Naba Bora, Grant Manager and Dr. Imes Beitins at the USAMRMC on the compliance of Human Subjects Protocols. Per Dr. Bora's instruction, we have prepared three new Human Subjects Protocols addressing all issues raised by the Regulatory Department. These protocols were submitted to the Institutional Review Boards (IRB) at The Ohio State University and have received approval for each protocol. The IRB approval letters have been sent to the Army for final approval of the compliance.

At least 30 vestibular schwannomas were previously procured by Dr. D. Bradley Welling (co-investigator). Six adjacent uninvolved vestibular nerves were also available. Written informed consent for tumors and nerves was obtained from all patients prior to surgical removal of their tumors. The current study does not pose any risks to the subjects. The specimens had all patient identifiers removed and were totally de-identified so that no one including the treating physicians and investigators could decode the samples to connect to patient's information. We have isolated RNAs from these frozen schwannomas and vestibular nerves.

Task 2: To prepare Schwann cell cultures, we consulted with Dr. Nancy Ratner at the University of Cincinnati. We visited her lab to learn the preparation of Schwann cell cultures from sciatic nerves. We are now ready to procure fresh vestibular nerves and sciatic nerves for Schwann cell cultures according to their protocol (Ratner et al., 1986). In addition, we have obtained RNAs from various normal human tissues.

Task 3: We have conducted reverse transcription-polymerase chain reaction (RT-PCR) analysis using RNAs from 10 vestibular schwannomas. RT-PCR analysis was also performed using RNAs from various normal human tissues. By cloning and sequencing we examined the complete exon structures and relative frequency of alternatively spliced *Nf2* isoforms.

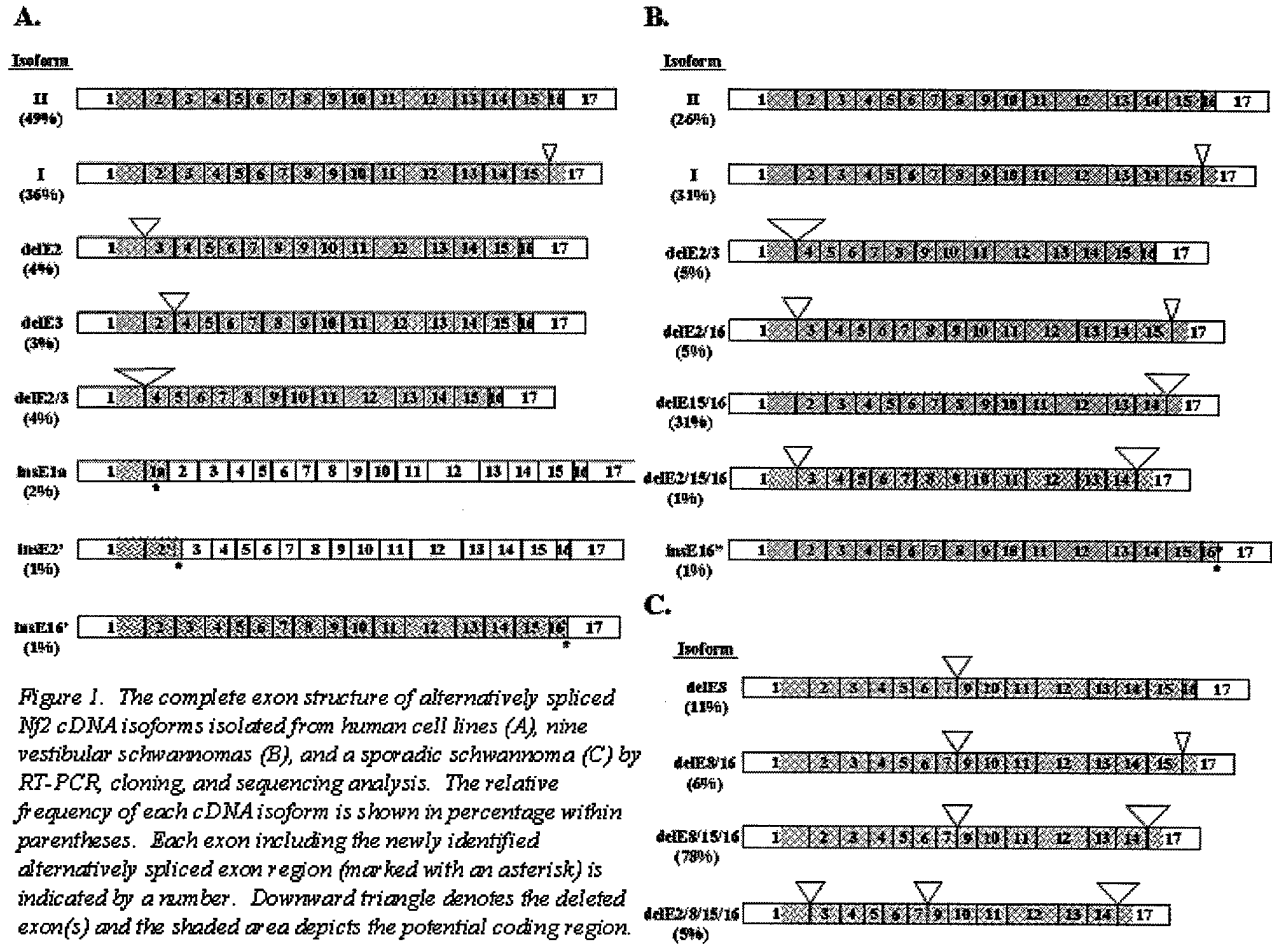

Figure 1. The complete exon structure of alternatively spliced Nf2 cDNA isoforms isolated from human cell lines (A), nine vestibular schwannomas (B), and a sporadic schwannoma (C) by RT-PCR, cloning, and sequencing analysis. The relative frequency of each cDNA isoform is shown in percentage within parentheses. Each exon including the newly identified alternatively spliced exon region (marked with an asterisk) is indicated by a number. Downward triangle denotes the deleted exon(s) and the shaded area depicts the potential coding region.

Previously we examined alternatively spliced *NF2* cDNA isoforms expressed in three human cell lines (293 kidney, SK-N-AS neuroblastoma, and NT2/D1 teratocarcinoma cells). We identified eight different alternatively spliced cDNAs (Figure 1A). Consistent to those reported previously, clones containing the *NF2* cDNA isoforms II (containing all 17 exons) and I (without exon 16) were the predominant

species.

We also examined alternatively spliced *NF2* isoforms in 10 schwannomas (1 NF2 schwannoma, 7 sporadic schwannomas, and 2 cystic schwannomas). Intriguingly, the expression pattern and relative frequency of the alternatively spliced *NF2* transcripts appeared to be different from those detected in other human cell types (Figure 1B). In addition to isoform I and II, these schwannomas expressed high percentage of the *NF2* mRNA isoform lacking exons 15 and 16. Isoforms with a deletion of exons 2 and 3, 2 and 16, and 2, 15 and 16 were also detected. In addition, an isoform with an insertion of a new exon 16", which contained additional nucleotides from intron 16 were obtained.

In addition, we identified a sporadic schwannoma, which predominantly expressed *NF2* transcripts lacking exons 8, 15 and 16 (Fig. 1C). Other cDNAs missing exon 8, exons 8 and 16, and exons 2, 8, 15, and 16 were also detected. Taken together, these results indicate that vestibular schwannomas express a distinct pattern of alternatively spliced *NF2* transcripts lacking specific exons.

We have also carried out a similar analysis of alternatively spliced *NF2* cDNA isoforms expressed in various normal human tissues. A total of 18 different *NF2* cDNAs were isolated (Table 1). Similar to those seen in human cell lines (Fig. 1A), isoforms I and II were the predominant species (Table 1). Isoform with a deletion of exons 15 and 16 also frequently detected in all five human tissues examined. The rest of *NF2* cDNA isoforms appeared to vary among various tissues.

Table 1. The alternatively spliced NF2 cDNA isoforms detected in various normal human tissues.

Tissue / *NF2* cDNA Isoform %	Placenta	Heart	Kidney	Brain	Fetal Liver
Isoform I	29%	24%	35%	34%	25%
Isoform II	29%	36%	34%	28%	31%
delE2	7%	4%		5%	2%
delE2/3		6%	3%	2%	
delE2/16			1%	1%	
delE2/15/16		6%			
delE2/3/16			1%		
delE2/3/15/16	4%		1%		
delE3/15/16	4%	3%	1%		2%
delE15		1%			
delE15/16	24%	20%	16%	30%	40%
delE8/16			1%		
delE8/15/16	24%		1%		
delE10			1%		
ins1a			2%		
ins1a/delE16			1%		
ins1a/delE15/16			2%		
ins1a/delE15	3%				

Task 4: We have isolated genomic DNA from the blood from the patient with a sporadic schwannoma that only expresses *NF2* mRNAs lacking at least exon 8. We are now in the process of conducting *NF2* mutational analysis on the patient's DNA.

Aim 2: Functional analysis of the two *NF2* isoforms commonly expressed in vestibular schwannomas.

Task 5: The two *NF2* isoform cDNAs (lacking exons 15 and 16 or exon 8 and 16) commonly expressed in vestibular schwannomas were cloned and placed under the control of a mouse myelin P0 promoter. Transgene fragments carrying the P0 promoter-driven each *NF2* cDNA are being prepared and will be used in the production of transgenic mice.

Task 6: To test the biochemical and biological activities of *NF2* cDNA isoforms, we have employed the pIND inducible expression system (Invitrogen). Hemagglutinin (HA) epitope-tagged *NF2* cDNA isoforms were constructed and inserted into the pIND vector. Cotransfection of the inducible vector carrying the HA-tagged *NF2* cDNA with the pVgRXR expression plasmid carrying a insect steroid hormone receptor into human 293 cells were performed.

Task 7: Upon induction of 293 cells cotransfected with an *NF2* cDNA expression vector and pVgRXR with an insect steroid hormone ponasterone, we detected the expression of the HA-tagged *NF2* isoform proteins using an anti-HA antibody. However, expression of the two schwannoma-expressed *NF2* isoform proteins did not give rise to any growth inhibitory effects in 293 cells.

To examine whether *NF2* mediated-growth inhibitory activity can only be detected in Schwann cell type, we are presently transfecting these *NF2* expression constructs into RT4 schwannoma cells.

Task 8: The two schwannoma-expressed *NF2* cDNA isoforms were fused with a GST-expression vector. These GST-*NF2* fusion vectors will be used to examine the ability of these *NF2* isoform proteins to form inter- and intra-molecular interaction.

Aim 3: Examination of the Potential Role of Differential Polyadenylation of *NF2* Transcripts.

Task 9: Three *NF2* cDNA expression plasmids carrying different 3'UT sequences were constructed and will be used in transfection studies to determine the half-life of *NF2* RNAs.

Task 10: Eight research abstracts were presented to national and local meetings.

KEY RESEARCH ACCOMPLISHMENTS

We have found that the pattern and relative frequency of alternatively spliced *NF2* isoforms expressed in vestibular schwannomas

appear to be different from those detected in other human cell types. Preliminary analysis showed that the two schwannoma-expressed *NF2* isoform cDNAs lacking exons 15 and 16 or exons 8 and 16 did not possess growth inhibitory activity in human 293 cells.

REPORTABLE OUTCOMES

Eight research abstracts were presented to national and local meetings (see Appendices)

CONCLUSIONS:

Vestibular schwannomas express a distinct pattern of alternatively spliced *NF2* transcripts lacking specific exons, suggesting that these alternatively spliced exons may be important for *NF2* function. Further experiments are in progress to address whether these alternative splicing *NF2* isoforms preferentially expressed in schwannomas possess any additional properties conducive to tumor formation *in vivo*.

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APPENDICES:

Abstracts Presented at National Meetings

(1) Chang, L.-S., E.M. Akhmametyeva, Y. Wu, and D.B. Welling. 2003. Transcriptional Regulation of the Human Neurofibromatosis 2 (*NF2*) Gene. Pediatric Academic Societies' Meeting, Seattle, WA.

(2) Akhmametyeva, E., S. Yao, Y. Wu, D.B. Welling, and L.-S. Chang. 2003. Novel Alternatively Spliced *NF2* Transcripts In Vestibular Schwannomas. Pediatric Academic Societies' Meeting, Seattle, WA.

(3) Welling, D.B., J.M. Lasak, E.M. Akhmametyeva, B.A. Neff, and L.-S. Chang. 2003. Analysis of Genes and Pathways Deregulated in Vestibular Schwannomas. The NNFF International Consortium for Molecular Biology of NF1 And NF2, Aspen, CO.

(4) Lebedeva, L., S.A. Trunova, N.A. Bulgakova, L.V. Omelyanchuk, E.M. Akhmametyeva, L.-S. Chang. 2003. The Role of *Drosophila* merlin in the Control of Mitosis Exit and Development. The NNFF International Consortium for Molecular Biology of NF1 and NF2, Aspen, CO.

(5) Welling, D.B., J.M. Lasak, E.M. Akhmametyeva, B.A. Neff, and L.-S. Chang. 2003. Analysis of Genes and Pathways Deregulated in Vestibular Schwannomas. 4th International Symposium on Vestibular Schwannomas and Other Cerebellum Pontine Angle Tumors, Cambridge, England.

Abstracts Presented at Local Meetings

(1) Akhmametyeva, E.M., Y. Wu, D.B. Welling, and L.-S. Chang. 2003. Analysis of Novel Alternatively Spliced *NF2* Transcripts in Vestibular Schwannomas. The Annual OSU Comprehensive Cancer Center Scientific Meeting, Columbus, OH.

(2) Chang, L.-S., E.M. Akhmametyeva, Y. Wu, and D.B. Welling. 2003. Transcriptional Regulation of the Human Neurofibromatosis 2 (*NF2*) Gene. Columbus Children's Research Institute Annual Research Conference, Columbus, OH.

(3) Akhmametyeva, E., Y. Wu, D.B. Welling, and L.-S. Chang. 2003. Novel Alternatively Spliced *NF2* Transcripts In Vestibular Schwannomas. Columbus Children's Research Institute Annual Research Conference, Columbus, OH.

4th International Symposium on Vestibular Schwannomas and Other Cerebellum Pontine Angle Tumors, Cambridge, England.

(Oral Presentation)

ANALYSIS OF GENES AND PATHWAYS DEREGULATED IN VESTIBULAR SCHWANNOMAS

Welling, D. Bradley, John M. Lasak, Elena M. Akhmametyeva, Brain A. Neff, and Long-Sheng Chang. *Departments of Otolaryngology and Pediatrics, The Ohio State University and Children's Hospital, Columbus, OH*

Background: Vestibular schwannomas are known to harbor mutations in the neurofibromatosis type 2 tumor suppressor gene (*NF2*), but the mechanism of *NF2* action is not well understood. Identification of genes differentially expressed in normal and diseased tissues using a large-scale cDNA microarray approach may lead to increased understanding of pathways leading to tumor formation.

Objective: The objective of this study was to evaluate the gene expression profiles in vestibular schwannomas when compared to normal vestibular nerve tissues, and to identify pathways, which may be altered in vestibular schwannomas.

Methods: Total RNA was extracted from one normal vestibular nerve and eight vestibular schwannomas. The normal vestibular nerve was from one of the seven patients with a small vestibular schwannoma. Radiolabeled cDNA was hybridized to cDNA microarray filters containing 25,920 known genes or expressed sequence tags (ESTs). Expression patterns were imaged and analyzed. Selected genes which showed three-fold or greater difference in the intensity between the normal nerve and the schwannomas were further examined by real-time PCR and by immunohistochemical staining.

Results: Forty-two genes (0.2%) were up regulated 3-fold or more in at least 5 of the 7 tumors when the filter images were compared to a normal adjacent vestibular nerve. Among them, osteonectin, an angiogenesis mediator, and RhoB GTPase, which is important in cell signaling, were significantly up regulated in 5 of 7 tumors. Among genes that were down regulated, an apoptosis-related LUCA-15 genes was highly under expressed in 6 of 7 schwannomas when compared to the normal nerve. Real-time PCR and immunohistochemistry data support the cDNA microarray findings. The retinoblastoma protein-cyclin dependent kinase (pRb-CDK) pathway was specifically evaluated and found to be frequently deregulated in vestibular schwannomas. Among genes in the pRb-CDK pathway, CDK2 was substantially under expressed in 7 of 8 tumors. Real-time PCR also showed consistent down regulation of CDK2 in the tumors. Anti-CDK2 antibody stained predominantly in the vestibular nerve and ganglion cells, but only weakly in the vestibular schwannoma tissues.

Conclusion: This cDNA microarray analysis of vestibular schwannomas suggests several interesting and potentially important tumorigenesis pathways associated with vestibular schwannoma formation. Further investigation into the regulatory mechanisms may lead to a better understanding of VS tumorigenesis.

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ABSTRACT FORM (Abstract not to exceed this frame)

TITLE: **ANALYSIS OF GENES AND PATHWAYS DEREGULATED IN VESTIBULAR SCHWANNOMAS**

Author(s): Welling, D. Bradley, John M. Lasak, Elena M. Akhmametyeva, Brain A. Neff, and Long-Sheng Chang
Position of Presenting Author: Professor

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Background: Vestibular schwannomas are known to harbor mutations in the neurofibromatosis type 2 tumor suppressor gene (*NF2*), but the mechanism of *NF2* action is not well understood. Identification of genes differentially expressed in normal and diseased tissues using a large-scale cDNA microarray approach may lead to increased understanding of pathways leading to tumor formation.

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ABSTRACT FORM (Abstract not to exceed this frame)

TITLE: **THE ROLE OF *DROSOPHILA* MERLIN IN THE CONTROL OF MITOSIS EXIT AND DEVELOPMENT**

Author(s): Lebedeva, Lidiya[1], Svetlana A. Trunova[1], Natalia A. Bulgakova[1], Leonid V. Omelyanchuk[1], Elena M. Akhmametyeva[2], and Long-Sheng Chang[2]

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The effect of a merlin mutation *mer4* (residue $170^{Gln\text{-}stop}$) on mitosis in the neural ganglia and the wing imaginal disk from the third instar larvae of *Drosophila* was examined. Cytological abnormalities at the anaphase and telophase stages were observed in the wing imaginal disc and the brain from the homozygous *mer4* larvae. Unlike the wild-type controls, the *mer4* mutant cells exhibited asynchronous anaphase; two sister chromosome sets showed different degrees of chromosome condensation. During normal mitosis, chromatin decondensation is a major characteristic of the late telophase. Instead, the *mer4* mutant cells frequently showed chromosome decondensation at anaphase.

Adult hemizygoius *mer4* males, while rare, were obtained after balancing the initial *mer4* stock with Binsn (Binsn = $In(1)sc^{S1L}sc^{8R}$+dl-49, sc^{8} sc^{S1} sn^{X2} B^{1}). Cytological analysis of testis cells from the hemizygoius *mer4* males during meiosis showed polyploidy at the onion-stage spermatides, suggesting a result of meiotic nondysjunction. Genetic analysis on the heterozygous *mer4*/FM7 females (FM7 = FM7, y^{31d} sc^{8} w^{a} v^{Of} B^{1} GFP) revealed sex chromosome nondysjunction in meiosis at the frequency of 1%. Introduction of an additional CyO inversion into the mer^{4}/FM7 genome further increased the frequency of sex chromosome nondysjunction to 7%. The latter results are in agreement with the distributive pairing rules of Grell (Grell, R.F. 1976. Distributive pairing. In *Genetics and Biology of Drosophila.* Eds. Ashburner, Novitski, Vol.1a, pp435—486) and may be explained by that the merlin mutation causes some levels of chromosome nondysjunction in a semi-dominant fashion.

Morphological analysis revealed that the hemizygous *mer4* males had some truncation in the first and second leg segments, appearing as unusual angled segments. In addition, abnormal shape and bristle pattern of wings were seen. These results suggest that merlin may have a regional effect on the extracellular matrix and/or cytoskeleton in certain tissues during *Drosophila* development. Consistent with this notion, the wing imaginal discs from the hemizygous *mer4* larvae, when cultured at 18°C, frequently showed outgrowths anterior or posterior to the proper wing blade. Taken together with previous reports by Fehon and colleagues, our results indicate that merlin is not only required for the regulation of cell proliferation but also important in the control of mitosis exit and development.

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Filename: 753775

Subspecialty: Oncology (see also Hematology)

Theme: Genetic Basis of Disease

2003 Pediatric Academic Societies' Meeting
Abstract Submission Form

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Keywords: Neurofibromatosis type 2; Transcription; Transgenic Mice
Is First Author a Trainee? No, Not a Trainee

Transcriptional Regulation of the Human Neurofibromatosis 2 (*NF2*) Gene

Long-Sheng Chang [1,2], Elena M. Akhmametyeva [1], Yong Wu [1] and D. Bradley Welling [2]. (Sponsored by Philip R. Johnson)[1], Columbus Children's Research Institute and Department of Pediatrics, The Ohio State University, Columbus, OH and [2]Department of Otolaryngology, The Ohio State University, Columbus, OH.

Background: Vestibular schwannomas, are the most prevalent tumor seen in neurofibromatosis type 2. Vestibular schwannomas are known to harbor mutations in the *NF2* gene; however the mechanism by which *NF2* inactivation causes tumor formation in Schwann cells of the vestibular nerve remains to be determined.
Objective: To better understand the regulation of the *NF2* gene and its role in vestibular schwannoma tumorigenesis.
Design/Methods: A 2.4-kb human <I style="mso-bidi-font-style: normal">NF2 promoter-driven luciferase gene and its 5' promoter deletion derivatives were generated. Site-directed mutagenesis was used to create point mutations in the <I style="mso-bidi-font-style: normal">NF2 regulatory elements. Subconfluent cells were transfected with various <I style="mso-bidi-font-style: normal">NF2 promoter constructs and transfected cell extracts were assayed for reporter enzyme activities. Band-shift assays were performed to detect specific DNA binding proteins. A 2.4-kb *NF2* promoter-driven b-galactosidase (b-gal) construct was used in transgenic mouse production. Transgenic embryos at various days post coitus were obtained and processed for X-gal staining.
Results: Transient transfection analysis in various human cells employing <I style="mso-bidi-font-style: normal">NF2 promoter-luciferase chimeric constructs revealed a core promoter region extending 400 bp from the major transcription initiation site. While multiple regions are required for full promoter activity, site-directed mutagenesis experiment identified a G/C-rich sequence, which could be bound by transcription factor Sp1, as a positive *cis*-acting regulatory element. Cotransfection studies in Drosophila SL2 cells showed that Sp1 could activate the <I style="mso-bidi-font-style: normal">NF2 promoter through the G/C-rich sequence. Transgenic mice carrying a 2.4-kb *NF2* promoter-

driven b-gal construct were produced. Whole-mount X-gal staining of transgenic mouse embryos detected transgene-encoded b-gal activity in several tissues including the brain. **Conclusions:** Our results indicate that the human *NF2* gene is regulated at the transcriptional level and suggest that such regulation may be important for *NF2* expression in Schwann cells during development. (Supported by the US Dept. of Defense Neurofibromatosis Research Program)

Disclosure: No information to disclose

First Author Signature:

Long-Sheng Chang, Ph.D.

Sponsor Signature:

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<SPAN>Novel Alternatively Spliced *NF2* Transcripts In Vestibular Schwannomas

Elena M Akhmametyeva [1], Shounan Yao [2], Yong Wu [1], D Bradley Welling [2] and Long-Sheng Chang [1,2]. (Sponsored by Philip R Johnson)[1], Columbus Children's Res Inst & Dept of Pediatrics, The Ohio State Univ, Columbus, OH and [2]Dept of Otolaryngology, The Ohio State Univ, Columbus, OH.

Background: Inactivation of human *NF2* gene is associated with neurofibromatosis type 2 (NF2), an autosomal dominant disorder with the hallmark being the development of bilateral vestibular schwannomas. The *NF2* gene is transcribed into multiple mRNAs via alternative splicing. Presently, the role of alternative splicing of *NF2* transcripts is not understood.
Objective: To examine the patterns of alternatively spliced *NF2* transcripts in vestibular schwannomas and other cell types
Design/Methods: RNAs were prepared from ten vestibular schwannomas and various normal human tissues and cell lines. RT-PCR was performed using a set of *NF2*-specific primers covering the entire coding region. The cDNAs were cloned and sequenced. The complete exon composition of *NF2* cDNAs and their relative frequency and encoded amino acid sequences were deduced
Results: Eight alternatively spliced *NF2* isoforms were isolated from normal brain, kidney, lung, and liver, and three cell lines. Isoforms II (with all 17 exons) and I (without exon 16) were the predominant species. Isoforms with a deletion of exon 2, 3, or 2/3 were detected at a modest frequency. Intriguingly, the pattern of alternatively spliced *NF2* transcripts and their relative frequency detected in vestibular schwannomas were different from those in normal tissues and cell lines. In addition to isoforms I and II, schwannomas expressed high percentage of the isoform lacking exons 15/16. Isoforms with a deletion of exons 2/3, 2/16, and 2/15/16 were also detected. Additionally, we identified a schwannoma which preferentially expressed the isoform lacking exons 8/15/16. Other isoforms missing exon 8, 8/16, and 2/8/15/16 were also detected at a lower frequency. Sequence comparison revealed

the *NF2* cDNAs from this schwannoma completely matched the wild-type *NF2* sequence with the exception of the spliced exons, whereas mutations were identified in the *NF2* cDNAs from other schwannomas. Experiments are in progress to examine the functional activity for each of these *NF2* isoforms.
Conclusions: Vestibular schwannomas express a distinct pattern of alternatively spliced *NF2* transcripts lacking specific exons, suggesting that alternative splicing may be an alternative mechanism for Schwann cells to inactivate *NF2* function and/or to generate isoforms that have additional properties conducive to tumor formation. (Supported by the US Department of Defense Neurofibromatosis Research Program)

Disclosure: No information to disclose

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ANALYSIS OF NOVEL ALTERNATIVELY SPLICED *NF2* TRANSCRIPTS IN VESTIBULAR SCHWANNOMAS. Elena M. Akhmametyeva[1,2], Yong Wu[1,2], D. Bradley Welling[3,4], and Long-Sheng Chang[1,2,3,4] *Columbus Children's Research Institute[1], and Departments of Pediatrics[2] and Otolaryngology[3], Molecular Biology and Human Cancer Genetic Program[4], The Ohio State University Comprehensive Cancer Center, Columbus, OH*

Inactivation of human neurofibromatosis 2 gene (*NF2*) is associated with neurofibromatosis type 2 (NF2), a highly penetrant, autosomal dominant disorder with the hallmark being the development of bilateral vestibular schwannomas. The *NF2* gene is transcribed into multiple mRNAs via alternative splicing and generates multiple mRNA isoforms lacking one or more exons. Presently, the role of alternative splicing of *NF2* mRNAs is not understood. The objective of this study is to examine and compare the patterns of alternatively spliced *NF2* transcripts in vestibular schwannomas and other cell types. By reverse transcription-polymerase chain reaction, cloning and sequencing, we identified eight different alternatively spliced *NF2* cDNA isoforms from normal human tissues (brain, kidney, lung, and liver) or cell lines of non-Schwann cell origin (NT2/D1, SK-N-AS, and 293). Isoforms II (with all 17 exons) and I (without exon 16) were the predominant species. Isoforms with a deletion of exon 2, 3, or 2/3 were detected at a modest frequency, were found at a low frequency. Intriguingly, we found that the pattern of alternatively spliced *NF2* transcripts and their relative frequency detected in vestibular schwannomas were different from those in normal tissues and cell lines. In addition to isoforms I and II, schwannomas expressed high percentage of the isoform lacking exons 15/16. Isoforms with a deletion of exons 2/3, 2/16, and 2/15/16 were also detected. Additionally, we identified a schwannoma which preferentially expressed the isoform lacking exons 8/15/16. Other isoforms missing exon 8, 8/16, and 2/8/15/16 were also detected at a lower frequency. Sequence comparison revealed the *NF2* cDNAs from this schwannoma completely matched the wild-type *NF2* sequence with the exception of the spliced exons, whereas mutations were identified in the *NF2* cDNAs from other schwannomas. In conclusion, vestibular schwannomas express a distinct pattern of alternatively spliced *NF2* transcripts lacking specific exons, suggesting that alternatively spliced exons may be important for *NF2* function. Our results further suggest that alternative splicing may be an alternative mechanism for Schwann cells to inactivate *NF2* function and/or to generate isoforms that have additional properties conducive to tumor formation. Experiments are presently in progress to examine the functional activity for each of these *NF2* isoforms.

Primary Program Affiliation: Molecular Biology and Human Cancer Genetics

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COLUMBUS CHILDREN'S RESEARCH INSTITUTE ANNUAL RESEARCH CONFERENCE May 29 and 30, 2003

ABSTRACT RECEIPT DEADLINE: 8:00 am, Monday, May 12, 2003

Novel Alternatively Spliced *NF2* Transcripts In Vestibular Schwannomas. Elena Akhmametyeva[1,2], Yong Wu[1,2], D. Bradley Welling[3], and Long-Sheng Chang[1,2,3] [1]Center for Childhood Cancer, *Columbus Children's Research Institute& Departments of* [2]*Pediatrics and* [3]*Otolaryngology, The Ohio State University, Columbus, OH*

Background: Inactivation of human neurofibromatosis 2 gene (*NF2*) is associated with neurofibromatosis type 2, a highly penetrant, autosomal dominant disorder with the hallmark being the development of bilateral vestibular schwannomas. The *NF2* gene is transcribed into multiple mRNAs via alternative splicing and generates multiple mRNA isoforms lacking one or more exons. Presently, the role of alternative splicing of *NF2* mRNAs is not understood.

Objective: The objective of this study is to examine and compare the patterns of alternatively spliced *NF2* transcripts in vestibular schwannomas and other cell types.

Methods: RNA was prepared from ten vestibular schwannomas and various normal human tissues and cell lines. Reverse transcription-polymerase chain reaction was performed using *NF2* specific primers covering the entire coding region. The cDNAs were cloned and sequenced. The complete exon composition of *NF2* cDNAs and their relative frequency and encoded amino acid sequences were deduced and compared.

Results: Eight different alternatively spliced *NF2* cDNA isoforms were isolated from normal human brain, kidney, lung, and liver, and three human cell lines. Isoforms II (with all 17 exons) and I (without exon 16) were the predominant species. Isoforms with a deletion of exon 2, 3, or 2/3 were detected at a modest frequency, were found at a low frequency. Intriguingly, the pattern of alternatively spliced *NF2* transcripts and their relative frequency detected in vestibular schwannomas were somewhat different from those in normal tissues and cell lines. In addition to isoforms I and II, schwannomas expressed high percentage of the isoform lacking exons 15/16. Isoforms with a deletion of exons 2/3, 2/16, and 2/15/16 were also detected. Additionally, we identified a schwannoma which preferentially expressed the isoform lacking exons 8/15/16. Other isoforms missing exon 8, 8/16, and 2/8/15/16 were also detected at a lower frequency. Sequence comparison revealed the *NF2* cDNAs from this schwannoma completely matched the wild-type *NF2* sequence with the exception of the spliced exons, whereas mutations were identified in the *NF2* cDNAs from other schwannomas.

Conclusion: Vestibular schwannomas express a distinct pattern of alternatively spliced *NF2* transcripts lacking specific exons, suggesting that alternatively spliced exons may be important for *NF2* function. Our results further suggest that alternative splicing may be an alternative mechanism for Schwann cells to inactivate *NF2* function and/or to generate isoforms that have additional properties conducive to tumor formation.

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ABSTRACT RECEIPT DEADLINE: 8:00 am, Monday, May 12, 2003

Transcriptional Regulation of the Human Neurofibromatosis 2 (*NF2*) Gene. **Long-Sheng Chang[1,2,3], Elena Akhmametyeva[1,2], Yong Wu[1,2], and D. Bradley Welling[3]**
[1]Center for Childhood Cancer, Columbus Children's Research Institute, & Departments of [2]Pediatrics and [3]Otolaryngology, The Ohio State University, Columbus, OH

Background: Vestibular schwannomas, the most prevalent tumor seen in neurofibromatosis type 2, continue to cause significant morbidity in spite of improvement in diagnosis and treatment. Understanding the underlying molecular mechanisms of tumor pathogenesis will lead to improved diagnostics and the development of improved therapeutics by which genetic manipulation or prevention of tumor growth at the cellular level may be possible, thereby reducing the associated morbidity. Vestibular schwannomas are known to harbor mutations in the *NF2* gene; however the mechanism by which *NF2* inactivation causes tumor formation in Schwann cells of the vestibular nerve remains to be determined.

Objective: The ultimate goal of this study is to better understand the regulation of the *NF2* gene and its role in vestibular schwannoma tumorigenesis.

Methods: A 2.4-kb human *NF2* promoter was fused with the luciferase expression cassette and a series of 5' unidirectional promoter deletions were created. Site-directed mutagenesis was used to create point mutations in the *NF2* regulatory elements. Subconfluent cells were transfected with various *NF2* promoter constructs and transfected cell extracts were assayed for reporter enzyme activities. Band-shift assays were performed to detect specific DNA binding proteins. A 2.4-kb *NF2* promoter-driven β-galactosidase (β-gal) construct was used in transgenic mouse production. Transgenic embryos at various days post coitus were obtained and processed for X-gal staining.

Results: Transient transfection analysis in various human cells employing *NF2* promoter-luciferase chimeric constructs revealed a core promoter region extending 400 bp from the major transcription initiation site. While multiple regions are required for full promoter activity, site-directed mutagenesis experiment identified a G/C-rich sequence, which could be bound by transcription factor Sp1, as a positive *cis*-acting regulatory element. Cotransfection studies in Drosophila SL2 cells showed that Sp1 could activate the *NF2* promoter through the G/C-rich sequence. Transgenic mice carrying a 2.4-kb *NF2* promoter-driven β-gal construct were produced. Whole-mount X-gal staining of transgenic mouse embryos detected transgene-encoded β-gal activity in several tissues including the brain.

Conclusion: Our results indicate that the human *NF2* gene is regulated at the transcriptional level and suggest that such regulation may be important for *NF2* expression in Schwann cells during development.